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THE CRITICAL ROLE OF ADENINE NUCLEOTIDE TRANSLOCASE 4 (ANT4) IN MALE REPRODUCTIVE FUNCTION: INSIGHTS FROM A MOUSE MODEL

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Adenine nucleotide translocator 4 (ANT4) is an ATP/ADP transporter crucial for male fertility, yet its comprehensive role in reproductive function remains unclear. This study utilized ANT4 knockout mice to elucidate the effects of ANT4 deficiency on male reproduction. We observed that ANT4 deficiency led to infertility and impaired testicular development. Further investigation revealed significant alterations in testicular oxidative stress, autophagy, and inflammation. Specifically, ANT4 loss resulted in an imbalance between oxidation and antioxidants, affected autophagic flux and the AKT-AMPK-mTOR signaling pathway, and increased pro-inflammatory factor expression. These findings underscore ANT4's importance in regulating oxidative stress, autophagy, and inflammation in testicular tissues, providing a nuanced understanding of its significance in testicular development and male fertility.

Keywords: adenine nucleotide translocator 4 (ANT4), male reproduction, oxidative stress, autophagy, inflammation, mitochondrial function, spermatogenesis, mouse model.

Spermatogenesis is an intricate process requiring coordinated support from multiple biological pathways. Central to this complex development is the availability of adequate energy and a homeostatic environment within the seminiferous tubules [1]. Adenine nucleotide translocator 4 (ANT4), an ATP/ADP transporter expressed in the early phases of spermatogenesis, has been identified as crucial for male fertility. Previous studies have shown that male mice lacking ANT4 exhibit early meiotic arrest at the leptotene spermatocyte stage, increased germ cell apoptosis, and complete infertility. However, the mechanisms through which ANT4 regulates testicular development and germ cell quality control remain to be fully elucidated [2].

This study aims to delineate the effects of ANT4 deficiency on male reproduction by focusing on three key aspects: oxidative stress, autophagy, and inflammation. These interconnected processes are critical for maintaining the optimal environment for spermatogenesis and are likely influenced by ANT4's role in mitochondrial function and energy metabolism.

The results of our study on ANT4 knockout mice revealed a comprehensive impact on male reproductive function. ANT4^{-/-} male mice exhibited complete infertility, accompanied by significantly reduced testicular weight and size, and a dramatic decrease in sperm count in the caudal epididymis. Histological examination using H&E staining showed severe atrophy of seminiferous tubules and extensive damage to the germinal epithelium structure, with a marked reduction in spermatogenic cells. Ultrastructural analysis via TEM uncovered significant mitochondrial abnormalities in ANT4^{-/-} mouse testes, including swelling, loss of cristae, and accumulation of autophagosomes. The knockout mice also displayed increased oxidative stress, evidenced by elevated levels of reactive oxygen species (ROS) and hydrogen peroxide (H₂O₂), along with altered expression of oxidative stress markers such as SOD1, CAT, and GPX1. Autophagy dysregulation was apparent through changes in the expression of autophagy-related proteins (LC3B, P62) and disruption of the AKT-AMPK-mTOR signaling pathway. Furthermore, an enhanced inflammatory response was observed, characterized by increased expression of pro-inflammatory factors (IL-6, IL-1 β) and activation of the NF- κ B signaling pathway. Proteomic analysis complemented these findings by revealing differential expression of proteins involved in peroxisome and glutathione metabolism in ANT4^{-/-} mice [fig 1]. Collectively, these results demonstrate the crucial role of ANT4 in maintaining testicular homeostasis and proper spermatogenesis, highlighting its importance in regulating oxidative stress, autophagy, and inflammation in male reproductive tissues.

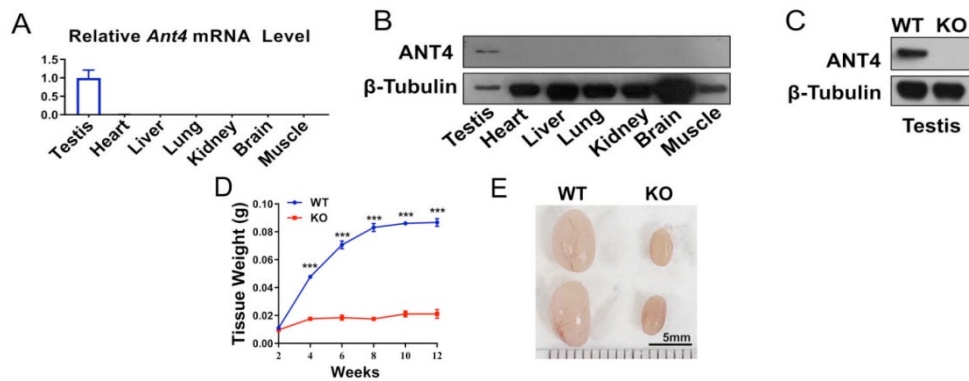


Fig.1 - Loss of *Ant4* results in male sterility. A, mRNA expression of *Ant4* in different tissues (n=3). B, Protein expression of ANT4 in different tissues. C, Protein expression of ANT4 in testes of wild type (WT) and *Ant4*^{-/-} knockout (KO) mice. D, Testicular growth curves (n=3). E, testis size

Conclusion: Our study demonstrates that ANT4 plays a multifaceted and essential role in male reproductive function. By coordinating cellular energy metabolism, protein quality control, redox balance, and inflammatory responses, ANT4 ensures the optimal environment for spermatogenesis. The loss of ANT4 leads to a complex interplay of disrupted cellular processes, ultimately resulting in severe impairment of male fertility.

These findings not only advance our understanding of the molecular mechanisms underlying male reproductive function but also provide potential new targets for the diagnosis and treatment of male infertility. Future research should focus on elucidating the specific molecular interactions of ANT4 with key regulators of oxidative stress, autophagy, and inflammation in testicular tissues.

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